THE INVENTION

The present invention discloses a previously unknown factor called Connective Tissue Growth Factor (CTGF), which is related immunologically and biologically to Platelet Derived Growth Factor (PDGF). Various cell types produce and secrete PDGF and PDGF-related molecules. In an attempt to identify the type of PDGF dimers present in the growth media of cultured endothelial cells, a new growth factor was discovered.

CTGF appears to play a role in the development, growth and repair of normal human tissue. The discovery of the CTGF protein and cloning of the cDNA encoding this molecule is significant in that it is a previously unknown growth factor having mitogenic and chemotactic activities in connective tissue cells. Although the biological activity of CTGF is similar to that of PDGF, CTGF is the product of a gene unrelated to the A or B chain genes of PDGF.

Since CTGF is produced by endothelial and fibroblast cells, both of which are present at the site of a wound, it is believed that CTGF functions as a growth factor in wound healing. Pathologically, CTGF may be involved in diseases in which there is an overgrowth of connective tissue cells, such as cancer, fibrotic diseases and atherosclerosis.

The primary biological activity of CTGF polypeptide is its mitogenicity, or ability to stimulate target cells to proliferate. The ultimate result of this mitogenic activity *in vivo*, is the growth of target tissue. CTGF also possesses chemotactic activity, which is the chemically induced movement of cells as a result of interaction with particular molecules. The CTGF of this invention is mitogenic and chemotactic for connective tissue cells, however, other cell types may be responsive to CTGF polypeptide as well.

The CTGF polypeptide of the invention is characterized by existing as a monomer of approximately 36-38 kD molecular weight which is secreted by cells and is active upon interaction with a PDGF receptor in cells.

The Applicants successfully isolated and purified CTGF protein and cloned the nucleotide sequence encoding CTGF, both of which were previously unknown in the prior art.

REJECTIONS UNDER 35 U.S.C. §112

The specification is objected to under 35 U.S.C. §112, first paragraph, as failing to adequately teach how to make and/or use the invention, i.e., failing to provide an enabling disclosure. Claims 14-16 are rejected under 35 U.S.C. §112, first paragraph, for the reasons set forth in the objection to the specification. Applicants respectfully traverse these rejections.

The specification is objected to as allegedly not providing enablement for the production of antibodies, either monoclonal or polyclonal, that react with the protein of the invention or fragments thereof, but do not cross-react with platelet derived growth factor (PDGF). The production of polyclonal and monoclonal antibodies is routine using the current techniques available. An example of one approach to producing antibodies is provided in the specification on page 9, lines 10-20. These antibodies bind with CTGF polypeptide or fragments thereof, and not with PDGF.

In addition, two references, Kohler, *et al*. and Ausubel, *et al*., are provided on page 9 as further sources to aid in antibody production. Lines 18-20 specifically describe a routine screening technique which can be used to readily identify antibodies which react with CTGF, but not PDGF. For example, one could pass a

sample containing antibodies prepared against CTGF over a column with PDGF bound to it, in order to eliminate any PDGF-binding antibodies. The flow through could then be passed over a column containing CTGF and any antibody which binds is CTGF-specific. The Office Action places emphasis on the lack of enablement in producing CTGF specific antibodies, however, the key step to distinguish PDGF from CTGF antibodies is the screening process. One skilled in the art could easily distinguish between CTGF and PDGF reactive antibodies using routine screening methods which are available in the two cited references as described above. Thus, following the teachings of the specification one skilled in the art could produce, identify and isolate antibodies, monoclonal or polyclonal, which react with CTGF and not PDGF.

General methods for raising antibodies to synthetic peptides which are identical to a portion of the sequence of a larger protein molecule, for the purpose of developing antibodies that are specific for that peptide, are known to those of skill in the art. These peptides typically range in size from 10 to 30 amino acid residues. By choosing unique sequences within a protein, one can easily develop antibodies which react only with that protein. Applicants have identified such a unique sequence in CTGF which corresponds to amino acid residues 232-259 (See peptide GG105, Appendix A of the accompanying declaration). Applicants have produced goat antibodies using this specific sequence of CTGF. Similar antibodies have been produced that specifically recognize the PDGF sequence. Neither the CTGF specific antibodies nor the PDGF specific antibodies recognize (i.e., bind) the other protein. The PDGF peptide-specific antibodies were used by Applicants to show that the CTGF peptide was not a PDGF derivative. Without the knowledge of the CTGF amino acid sequence which was identified by Applicants, CTGF specific antibodies could not have been routinely produced.

Applicants have provided the results of a routine experimental protocol for the production of polyclonal antibodies which specifically bind CTGF, but not PDGF. This protocol is discussed in the accompanying declaration by Dr. Gary R. Grotendorst (see paragraph 5). Briefly, GG105, a 28 amino acid peptide (Figure 1, Appendix A), was used to immunize goats (50μg) in Freund's complete adjuvant by multiple intradermal injections. All subsequent immunizations were with 50 μg of peptide in Freunds incomplete adjuvant. Immune sera were collected seven days after the fourth challenge and subsequent challenges. Western blot analysis of the immune sera (Figure 2, Appendix B), showed that anti-CTGF antibody reacted well with CTGF but did not exhibit any cross-reactivity to PDGF. (Figure 2, Appendix B: Lane 1 shows molecular weight markers; Lane 2, COS7 cells transfected with the plasmid pcDNAI, expressing CTGF open reading frame in antisense orientation; Lane 3, COS7 cells transfected with pcDNAI, expressing CTGF open reading frame in sense orientation; Lane 4, Recombinant PDGF AB (10ng)). Panel A was probed with anti-PDGF IgG and Panel B was probed with anti-CTGF (GG105 peptide) antiserum. The results clearly show that the anti-PDGF IgG detects both PDGF and CTGF, as seen in Panel A, lanes 3 and 4. In sharp contrast, the anti-CTGF antisera detected only the CTGF-peptide (Panel B, lane 3) and not the PDGF peptide (Panel B, lane 4), demonstrating the specificity of the antibody.

Standard techniques for producing monoclonal antibodies are known in the art. Given the peptide sequence of CTGF, it would be a matter of routine to produce monoclonal antibodies which bind CTGF, but not PDGF. Given the routine methods of screening large numbers of hybridoma supernatants (e.g., binding of supernatant to plates with CTGF peptide but not binding to plates with PDGF), one could easily identify CTGF-specific monoclonal antibodies without undue experimentation.

Monoclonal antibodies have been produced that recognize only a single amino acid substitution in a peptide (i.e., *ras* oncogene protein). Thus, it is predictable that unique CTGF monoclonals can be produced that would not react with PDGF, as there is only a 10% sequence identity between the PDGF A chain and CTGF and a 17% sequence identity between the PDGF B chain and CTGF. Applicants have produced PDGF A and PDGF B chain specific antibodies and these two peptides share a 50% sequence identity overall. These data indicate that contrary to the statements in the Office Action, it would be extremely difficult to produce a monoclonal antibody to CTGF that would react with PDGF. Consequently, Applicants respectfully request that this rejection be withdrawn.

Claims 1, 4, and 14-16 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter Applicant regards as the invention. Applicants respectfully traverse this rejection.

The Office Action states that the claims remain indefinite for failing to adequately specify the protein of the invention. Claim 4 has been canceled. Claim 1, as amended, now includes unique properties associated with the polypeptide of the invention, i.e., the polypeptide and fragments of the polypeptide which are biologically active, are mitogenic and chemotactic with respect to connective tissue cells; the polypeptide has a molecular weight of approximately 36 kD by non-reducing SDS-PAGE and approximately 38 kD by reducing SDS-PAGE; binding to a PDGF receptor; existing as a monomer; and having a polypeptide sequence according to Sequence ID No. 2, and conservative variations thereof. These properties are not possessed by any other polypeptide known to Applicants.

The Office Action also states that the term "fragments" renders claim 1 indefinite. Page 5, lines 9-15 of the specification recites that "CTGF polypeptide includes functional fragments of the polypeptide, so long as the mitogenic and chemotactic activities of CTGF are retained. Smaller peptides containing the biological activity of CTGF are included in the invention." Example 2, pages 17-19 of the specification, provides a functional assay for CTGF activity which can be used routinely to evaluate a CTGF fragment to determine whether the fragment has the requisite biological activity. Applicants believe that these descriptions clearly define what is meant by the term "fragments".

The Office Action states that the amendment to claim 14 remains indefinite for failing to define "specifically bind". Applicants have amended claim 14 to state that the antibodies of the invention bind the CTGF polypeptide of Sequence ID No.2, but not platelet derived growth factor (PDGF). Claim 14 as amended more clearly defines the antibodies of the invention.

The Office Action states that the use of the term "fragments thereof" in claim 14 fails to adequately define the metes and bound of that which applicant sees as the invention. Applicants believe that the amendment to claim 14 renders this objection moot.

REJECTIONS UNDER 35 U.S.C.§102

Claims 1 and 4 stand rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103 as obvious over Matsuoka, *et al.*, or alternatively Campochiaro, *et al.*, or alternatively Shimokado, *et al*. Applicants respectfully traverse this rejection. Claim 4 has been canceled.

Matsuoka, *et al*. disclose a family of PDGF-related proteins found in human wound fluid. These proteins were identified using a polyclonal antiserum to PDGF. Two peptide fractions, from 16-17 kD and 34-36 kD, were found to be immunoreactive with the polyclonal antibodies. The Office Action states that the 34-36 kD protein fraction appears to be identical to the CTGF of the present invention.

The mitogenic and chemotactic activities in Matsuoka correlated only with the 16-17 kD peptide(s), as shown in Figure 4, page 4419. The 34-36 kD fraction possessed no biological activity and was only present in trace amounts at the time when PDGF-like bioactivity was observed in wound fluid. On page 4418, column 2, lines 15-27, describe the correlation between the peptides described in the reference and biological activity. The kinetics of appearance and disappearance of the 16-17 kD and 34-36 kD product were independent of each other. According to the analysis of Figure 4B and 4C on page 4418, the Figure legend is mislabeled; the closed circles represent the total wound fluid and the open circles represent the immunopurified material. (See accompanying Declaration, paragraph 9.) Importantly, as stated in the discussion on page 4418, column 2, lines 15-27, the level of the 16-17 kD peptide peaked on the first day after surgery and decreased exponentially to nearly undetectable levels by the seventh day. In contrast, the 34-36 kD product was initially present at low levels and then increased, reaching peak levels on the fifth and sixth days postsurgery. The chemotactic and mitogenic activity of the total wound fluid and immunoabsorbed fraction show the highest activity on day 1 and decreased to undetectable levels by day 4, which correlates with the kinetics of appearance and disappearance of the 16-17 kD peptide.

Therefore, the biological activity found in Matsuoka, *et al.*, correlates with a 16-17 kD protein and not a 34-36 kD protein. In view of the failings of Matsuoka to teach or suggest the polypeptide of the invention, Applicants respectfully request that the rejections based on this reference be withdrawn.

Campochiaro, *et al.* disclose the isolation of a PDGF-like protein from retinal pigment epithelial cells. Western blot analysis using polyclonal anti-PDGF antiserum identified an 18.5 kD band and a band of 36-38 kD in retinal pigment epithelial cell conditioned media. Using an anti-PDGF IgG affinity column, bands with molecular weights of just less than 36 kD and 18 kD were found.

The proteins identified in Campochiaro, et al., were identified using polyclonal antiserum to PDGF. Therefore a family of PDGF-related proteins was identified, however, the individual proteins were not purified. The authors stated on page 221, line 1, that they found "multiple bands of 36-38 kD" and a band at 18.5 kD which possessed mitogenic and chemotactic activities. It is not clear what the antiserum is reacting with in the protein preparation. Proteins which share common amino acid sequences, or common primary structure, within an epitope, may react with the same antiserum. However, that does not mean that the proteins function similarly. In fact, the activity in Campochiaro appears to be due to another protein which was not even detected by Western blot analysis.

In addition, a 1-D gel, as was used in Campochiaro, would not be considered definitive of a purified protein. In the absence of 2-D gel electrophoretic analysis, it is not clear whether the "multiple bands" contain a single protein which possesses the biological activity of CTGF or several proteins.

On pages 225-226, in the last paragraph of the discussion, the authors state that "PDGF-like proteins from different cell types

may have structural differences that account for differences in migration, but retain important functional and antigenic similarities that warrant combining them under the heading of PDGF-like proteins." Without amino acid sequence comparison, it is scientifically incorrect to conclude that the proteins are the same. Therefore, the 36-38 kD protein(s) disclosed by Campochiaro, *et al.*, cannot be characterized as anything other than additional members of the PDGF-related protein family.

No real data on the structure of the protein can be deduced from Campochiaro, *et al.* For example, in the results section of the paper which describes Figure 5, more than one protein is seen in the anti-PDGF IgG purified ^{35}S-methionine fraction. <u>This shows that these fractions are not pure</u>. Furthermore, the size of the peptides detected in the western blot (> 36 kDa) are larger than those detected by autoradiography of the anti-PDGF IgG immunoabsorbed media (< 36 kDa) indicating that they are two different proteins. Additionally, the authors detected a peptide at 18.5 kDa which they assume suggests that the 36 kDa peptide is a dimer. This directly teaches away from the CTGF protein of the invention. CTGF is <u>not</u> a dimeric protein. It is a <u>monomer</u>. The authors suggest that the difference in size between the peptides detected by the western blot method versus the size of the radiolabled peptides is due to sample preparation. As co-author of the manuscript, Applicants do not believe this to be the case, but believe that the samples contained multiple proteins which were not pure. Consequently, the Applicants respectfully request that any rejections based on this reference be withdrawn.

Shimakado, *et al.*, disclose the isolation of a PDGF-like protein having a molecular weight of 37 kD, isolated from activated human alveolar and peritoneal macrophages. Two classes of PDGF-like

proteins which contained mitogenic activity and were antigenically similar to PDGF had molecular weights between 37-39 kD and 12-17 kD.

On page 281, column 1, lines 1-10 show that the mitogenic activity of the 14-17 kD fraction and the 37 kD fraction represent approximately 56% and 40%, respectively, of the mitogenic activity in the macrophage conditioned medium. The figure legend to Figure 6 states that the recovery is only 15-20% of the total activity loaded on the gel. Therefore, it is difficult to accurately determine how much activity is actually present in any one fraction and how much is attributable to one specific protein. In addition, none of the samples are pure. In the results section on page 280-281, Shimakado discuss how the gel filtration fraction II is not really a high molecular weight protein but is interacting with the Sephadex chromatography media and artifactually appearing to be 37 kDa. They also state that this fraction contains from 0.2-20 ug/ml of insulin. Furthermore, they demonstrate in Figure 7 that no CTGF could have been present, because after labelling cells with ^{35}S-cysteine and immunoprecipitation with an anti-PDGF antibody, no 37 kDa peptide is detected when the samples are run under reducing conditions.

In addition, the protein of Shimakado is 39 kD under non-reducing conditions (page 282, column 2) while the protein of the present invention is 36 kD under non-reducing conditions. The protein of Shimakado shifts from 39 kD under non-reducing conditions to 12 kD under reducing conditions (see discussion as above). Actually, under reducing conditions, Shimakado only finds <u>both</u> a 45 kD and a 12 kD protein (page 281, column 2). In contrast, the protein of the present invention migrates <u>as a single band</u> at approximately 38 kD under reducing conditions. Because CTGF's amino acid composition is over 10% cysteine, electrophoresis methods used by Shimakado would be very sensitive for detection of CTGF, yet none

was detected in the macrophage conditioned media. The fact that all of the Shimakado peptides that run at higher molecular weights under non-reducing conditions (Figure 7), run at 12 kDa under reducing conditions, demonstrates that these proteins are multimers of this lower molecular weight peptide. This conclusion is supported by the authors at page 281 in the last paragraph of the results section and is restated in the discussion section on page 282:

> "The 39,000 dalton ^{35}S-labeled band appeared to shift after reduction to approximately 12,000 daltons".

Therefore, the protein(s) of Shimakado do not migrate in a region which even remotely corresponds to the CTGF of the present invention. Thus, none of the Skimakado fractions were pure proteins and the characterization of the biological activity which was PDGF-related exhibited behavior on SDS gels which proving that it is not CTGF.

Additional proof that the peptides described by Shimakado cannot be CTGF is found in the accompanying declaration, paragraph 6, and Figure 3, Appendix C, where Applicants analyzed the expression of the CTGF gene in human umbilical vein endothelial cells, human skin fibroblasts, human neutrophils and human macrophages after activation with endotoxin. The fibroblasts expressed low levels of the CTGF gene and expressed higher levels after activation with TGF-β. Neutrophils and macrophages did not express detectable levels of the CTGF gene. Thus, it would be impossible for normal macrophages to make the CTGF protein if they do not produce the mRNA encoding this polypeptide. This confirms the data in Shimakado, *et al.* showing that there is no monomeric 37 kDa peptide containing cysteine that is secreted by the macrophage which could be detected with an anti-PDGF antibody. It is clear that the CTGF

of the present invention, and the proteins of Shimakado are distinct proteins. Consequently, Applicants respectfully request that any rejections based on this reference be withdrawn.

Claims 1 and 4 stand rejected under 35 U.S.C. §102 as anticipated by or, in the alternative, under 35 U.S.C. §103 as obvious over Ryseck, *et al.* While Applicants respectfully traverse this objection, it is believed that the accompanying declaration by Dr. Gary Grotendorst regarding submission of the CTGF sequence to GenBank renders the objection moot. The clone and sequence of CTGF were submitted to GenBank on July 17, 1990, prior to the May 1991 publication date of Rysek.

Claims 14-16 have been rejected under 35 U.S.C. §103 as being unpatentable over Matsuoka, *et al.*, or alternatively Campochiaro, *et al.*, or alternatively Shimokado, *et al.*, or alternatively Ryseck, *et al.* Applicants respectfully traverse this rejection.

As stated in the comments above, the CTGF of the present invention and the proteins identified in the above-cited references are distinguishable. In other words, antibodies which specifically bind CTGF and not PDGF could not have been produced prior to Applicants discovery of the CTGF which is required for immuniza-tion. As stated above, the references fail to teach or disclose CTGF as defined in the present invention. Therefore, since the CTGF of the present invention is a novel protein, antibodies which specifically bind to CTGF must also be novel. The antibodies used for isolating the proteins in the above-cited references were raised against PDGF and not CTGF. Those antibodies are not specific for CTGF as they recognize other growth factors, including PDGF AA, AB and BB and leukocyte-derived growth factor (LDGF). Therefore, the antibodies of the invention which bind CTGF and not PDGF are novel. The Applicants respectfully request that any rejections based on these references be withdrawn.

In summary, based on Applicants' amendments to the claims and comments above, it is respectfully submitted that claims 1, and 14-16 clearly and patentably define the invention. Applicants respectfully request that the Examiner reconsider the various grounds of rejection set forth in the Office Action and, in light of Applicants' response, allow the claims now pending to proceed to issuance.

Respectively submitted,

SPENSLEY HORN JUBAS & LUBITZ

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John R. Wetherell, Jr., Ph.D.
Reg. No. 31,678

1880 Century Park East
Suite 500
Los Angeles, CA 90067
(619) 455-5100
LAT/dn